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The DNA binding domains of the yeast Gal4 and human c-Jun transcription factors interact through the zinc-finger and bZIP motifs

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ABSTRACT

Different Gal4 fusion proteins, expressing unrelated transcription activator domains, were found to activate transcription from promoters containing dimerized AP1 DNA binding sites. Transactivation was dependent on the first 74 amino acids of Gal4. A direct interaction between Gal4 and c-Jun was demonstrated using a GSTGal4 fusion protein and *in vitro* translated human c-Jun. The interaction required the zinc finger containing DNA binding domain of Gal4 and the basic-leucine zipper region of c-Jun. These results demonstrated that the specificity of Gal4 fusion proteins in transient transfection experiments in mammalian cells is not restricted to reporters containing Gal4 binding sites, but also includes promoters containing AP1 binding sites. Furthermore, the Gal4 fusion proteins also activated transcription from a pUC18 vector fragment containing several putative AP1 binding sites. Finally, our results indicate that Gal4 activator proteins binding to Gal4 binding sites and to DNA bound AP1 factors can co-operatively activate transcription.

INTRODUCTION

A prototypic transcription factor contains a sequence specific DNA binding domain that confers promoter specificity and a transactivating domain mediating the interaction with the basal transcription machinery. The two domains can be targets for independent regulation by post-translational modifications or interaction with additional factors. Furthermore, combinatorial interactions between related and unrelated transcription factor subunits provide additional levels of regulation of promoter activity.

Most transcription factors bind as dimers to palindromic *cis*-acting DNA binding elements and can be classified according to the structure of their DNA binding domains and dimerization motifs. The AP1 family of transcription factors belongs to the bZIP protein family (1) and contains Jun and Fos related proteins. Specific DNA binding of AP1 is mediated by a highly basic amino acid sequence followed by a leucine zipper dimerization motif (2–5). The AP1 family of transcription factors is heterogeneous and consists of Jun/Jun homodimers and heterodimers between members of the Fos and Jun subgroups (reviewed in 6). The Jun proteins can also heterodimerize with members of the CREB/ATF family, which also contains a bZIP DNA binding motif (7 and references therein). This confers additional combinatorial heterodimerization possibilities (8) and hence a possible cross-talk at the transcriptional level between distinct signal transduction pathways.

The complexity is further increased by the observation that AP1 transcription factors can interact with members of other transcription factor families containing unrelated DNA binding motifs. For example, several steroid hormone receptors inhibit AP1 dependent transcription and reciprocally, AP1 factors can inhibit activation of steroid hormone responsive genes (9–13). The DNA binding domain of the steroid hormone receptors consists of a zinc finger region (reviewed in 14). No cross-reactivity in DNA binding specificity between AP1 and steroid hormone receptor proteins have been observed. Instead, mutational analyses have shown that the DNA binding domain of the glucocorticoid receptor (GR) (11,12,15) and the dimerization motif of the AP1 factor (11,13) are necessary for the inhibitory activity . A direct interaction between Jun/Fos and GR has also been detected both *in vivo* and *in vitro* (9,12,13,16). The bZIP domain of Jun has also been implicated in transcriptional repression through a putative interaction with other, unrelated transcription factors, such as MyoD (17), factors recognizing the insulin enhancer (18) and NF-IL6 (19). Collectively, these examples suggest that Jun (and possibly other members of the AP1 family) represents a common factor serving as a link between different signal transduction pathways.

The yeast transcription factor Gal4 binds to a specific palindromic sequence that has, so far, not been found in any promoters of mammalian genes. Therefore, it is generally assumed that Gal4 lacks a mammalian homologue. Thus, fusion proteins containing the Gal4 DNA binding domain have been widely used to study the function of transcription activation domains on reporter constructs carrying Gal4 binding sites.

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However, the Gal4 DNA binding domain contains a zinc finger structure with features similar to the zinc finger domains of transcription factors belonging to the steroid hormone receptor family (14,20 and references therein). In this paper we show that several Gal4 fusion proteins activate transcription from promoters containing AP1 DNA binding sites but lacking Gal4 DNA binding sites. We also demonstrate that the zinc finger domain of Gal4 can interact with the bZIP domain of c-Jun. We propose that this interaction is a productive interaction *in vivo*, and can lead to the tethering of an activating domain to promoters containing AP1 binding sites.

MATERIALS AND METHODS

Plasmid DNA

G1E1BCAT, E4CAT, $(E2F)_4$CAT and E1BTATACAT have all been described previously (21,22). collTRECAT and E3TRECAT were constructed by insertion of synthetic oligonucleotides, containing dimerized AP1 binding sites derived from the collagenase (tcgacCTGACTCATACTGACTCAta) or adenovirus E3 (tcgacCTTAGTCATACTTAGTCAta) promoters, into *SalI*/*Hin*dIII digested E1BTATACAT. In G1E1BCATΔ, the *PvuI*/*Hin*dIII fragment (from within the ampicillin gene to immediately upstream of the Gal4 DNA binding site) was replaced by the corresponding fragment from E1BTATACAT. pUC-E1BCAT contains a ~600 bp *XhoI*/*Hin*dIII fragment from G1E1BCAT inserted into the corresponding sites in E1BTATACAT.

LexA-v-junΔ9 contains the bacterial LexA protein fused to the DNA binding domain of v-jun (23). CMVc-jun expresses full-length human c-Jun (24). Gal4/CR1 and Gal4/CR3 express in frame fusion proteins between Gal4(1–147) and amino acids 1–90 or 121–192 from adenovirus E1A-289R protein, respectively (25). Gal4(1–74)/CR1 expresses the first 74 amino acids, and Gal4(79–147)/CR1 amino acids 79–147, of Gal4 fused to amino acids 1–90 of the adenovirus E1A protein.

Gal4/VP16 and Gal4/p53 have been described (26,27).

For *in vitro* translation purposes, the human c-jun cDNA from CMVc-jun was recloned into *Eco*RI/*Hin*cII digested SP65 vector (Promega), creating SP6hc-Jun. SP6hc-Jun-DBD was derived from SP6hc-Jun by deletion of the first 246 amino acids of c-Jun.

GSTGal4(1–94), GSTGal4(1–74), GSTGal4(74–147), GSTGal4 (1–147) are derivatives of the pGEX vectors (Pharmacia) and express fusions between the glutathione S-transferase (GST) protein and indicated amino acids of Gal4. In GSTGal4(1–147)/CR1 amino acids 1–90 from the adenovirus E1A protein is included in the fusion protein.

Cell culture conditions and transfection

Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% newborn calf serum (Hela cells) or 5% fetal calf serum (A14 cells), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Transfections were done in 60 mm Petri dishes by the calcium phosphate coprecipitation technique essentially as described in (28). Unless otherwise indicated the amount of transfected plasmids was 1–3 μg for the reporters, 0.5–2 μg for the Gal4 activators and 0.5 μg for CMVc-jun. The total amount of transfected plasmid DNA was adjusted to 9–12 μg with salmon sperm DNA. Cells were harvested at ~48 h post transfection and cell extracts prepared by three freeze–thawings in 0.25 M Tris–HCl, pH 7.5.

CAT assay

Chloramphenicol acetyl transferase assays were performed essentially as described (29). The results were quantitated using the ImageQuant computer program on a Phosphorimager (Molecular Dynamics).

Electrophoretic mobility shift assay

25 ng of a ^{32}P-end-labelled 70 bp fragment containing a dimerized AP1 binding site from the collagenase promoter was mixed with *in vitro* translated proteins essentially as described (30). Briefly, the binding was done at 4°C for 30 min in binding buffer (10 mM Hepes pH 7.6, 50 mM NaCl, 4 mM $MgCl_2$, 0.1 mM EDTA, 4 mM spermidine, 2 mM DTT, 0.1 μg/μl BSA, 54 ng/μl polydI–dC, 15% glycerol) before loading on a 4.25% native polyacrylamide gel. Electrophoresis buffer was 0.25 × TBE (22 mM Tris, 22 mM Boric acid and 0.5 mM EDTA, pH 8.0) and the gel was run at 4°C at 200 V for 3.5 h.

Coupled *in vitro* transcription/translation

The commercially available TNT™ SP6 coupled wheat germ extract system from Promega was used according to the manufacturer's instructions. Five μl of the translation mix was used in the experiments. Full length c-Jun and c-Jun-bZIP were synthesized directly from plasmids pSP6hc-Jun and pSP6hc-JunDBD, respectively. c-Jun-TAD was synthesized from pSP6hc-Jun, which had been linearized at the *Bst*XI site, resulting in a truncation of the c-Jun protein after amino acid 244.

GST binding assay

GST fusion proteins were produced in *E.coli* and bound to glutathione agarose beads (Current Protocols in Molecular Biology). Protein concentrations were estimated on a Coomassie stained SDS–polyacrylamide gel. Approximately equal amounts of GST fusion proteins were mixed with 5 μl ^{35}S methionine labelled *in vitro* translated proteins in WCEΔ buffer (25 mM Hepes, pH 7.6, 75 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.025% Triton X-100, 0.5 mM DTT) and incubated rotating at 4°C for 3 h. Beads were washed four times in Hepes binding buffer (20 mM Hepes pH 7.6, 50 mM NaCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA, 0.05% Triton X-100) and bound proteins were separated on a polyacrylamide gel at 150 V for 8 h and visualized by autoradiography.

RESULTS

A Gal4/CR1 fusion protein activates transcription through AP1 DNA binding sites

Fusion proteins between putative *trans*-activator domains and the yeast Gal4 DNA binding domain have been widely used in studies of mammalian transcription factor function. Such studies are based on the assumption that the Gal4 DNA binding domain, *per se*, does not interact with endogenous transcription factors, but functions by directing the Gal4 fusion protein to a promoter through binding to its natural DNA binding element. However, the Gal4 DNA binding domain contains a zinc finger and a dimerization motif that could theoretically interact with other

proteins. We have previously shown that a Gal4 (amino acids 1–147) protein fusion, expressing the conserved region 1 (CR1) of the adenovirus E1A-243R protein (Gal4/CR1), activates transcription from a reporter plasmid driven by a synthetic promoter containing one Gal4 DNA binding site (G1E1BCAT; Fig. 1A) (25). As shown in Figure 1A, the Gal4/CR1 fusion protein also activated transcription from two reporters where the Gal4 DNA binding sites had been replaced by dimerized AP1 DNA binding sites. The strongest activation was observed on a reporter containing the AP1 DNA binding site from the collagenase promoter (collTRECAT; Fig. 1A), but a reporter containing the AP1 DNA binding site from the adenovirus E3 promoter was also significantly activated (E3TRECAT; Fig. 1A). In contrast, the E1BTATACAT, which is identical to collTRECAT and E3TRECAT but without inserted upstream binding sites, the E2F binding sites from the adenovirus E2 promoter and the complete adenovirus E4 promoter did not serve as targets for Gal4/CR1 transactivation (Fig. 1A).

The AP1 factors consist of different dimeric combinations between members of the Jun and Fos families (6), or Jun and the CREB/ATF family (7,31,32,33). A v-Jun mutant (LexA-v-Jun Δ9), which lacks the transactivation domain of Jun but retains the ability to bind an AP1 DNA binding site, will inhibit AP1 dependent polyoma virus transcription (23). Cotransfection of LexA-v-Jun Δ9 and a c-Jun expression vector (CMVc-jun) strongly reduced c-Jun activated expression from collTRECAT. Similarly, LexA-v-Jun Δ9 also reduced Gal4/CR1 transactivation of collTRECAT. In contrast, adenovirus E1A (Gal4/CR3) activated transcription from E4CAT, lacking AP1 DNA binding sites, was not affected by LexA-v-Jun Δ9 (Fig. 1B). Collectively, these results suggested that the Gal4/CR1 fusion protein activated transcription through an AP1 DNA binding site.

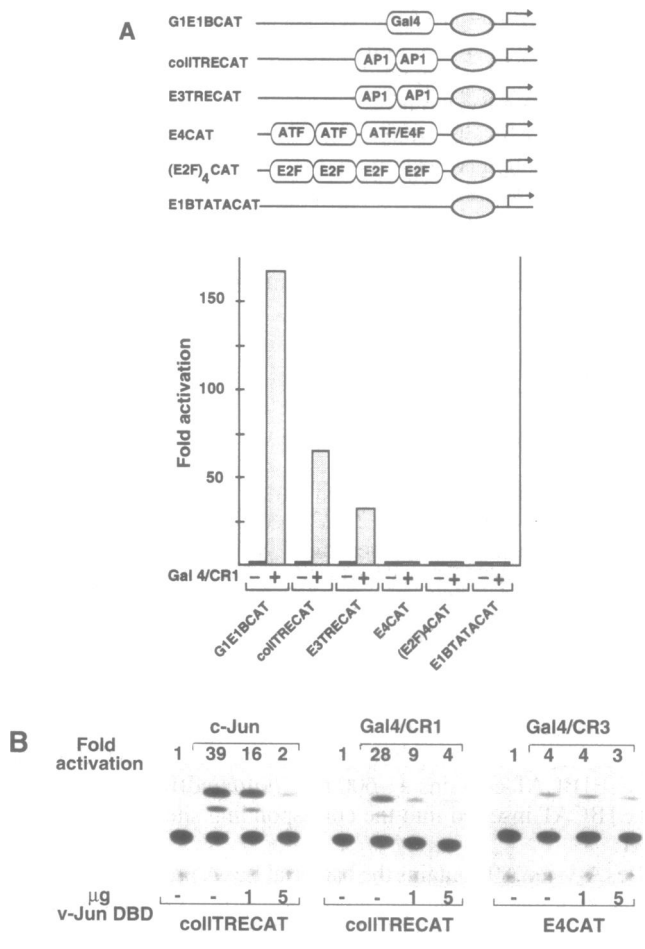

Figure 1. Gal4/CR1 activates AP1-driven transcription. (**A**) Hela cells were transfected with the CAT-reporter constructs depicted at the top, in the presence or absence of Gal4/CR1. Hatched circles represent adenovirus E1B TATA box. Upstream binding sites for cellular transcription factors are shown. The graph shows the mean value of induction of CAT activity from at least three independent experiments. (**B**) The DNA binding domain from v-Jun reduces Gal4/CR1 activation. collTRECAT and E4CAT were cotransfected with CMVc-Jun, Gal4/CR1 or Gal4/CR3, into Hela cells as indicated. Increasing amount of pLex A-v-jun Δ9 (v-JunDBD), a fusion construct between the bacterial LexA protein and the v-jun DNA binding domain, was included.

Several strong transactivating domains activate transcription from AP1 DNA binding sites when expressed as Gal4 fusion proteins

To exclude the possibility that transactivation through AP1 DNA binding sites specifically required the adenovirus E1A transactivation domain, two unrelated Gal4 fusion proteins were analysed. Gal4/VP16 and Gal4/p53 activated transcription from a reporter containing Gal4 binding sites, but not from a reporter lacking both Gal4 binding sites and AP1 DNA binding sites (26,27) (Fig. 2 and data not shown). Figure 2 shows that Gal4/VP16 and Gal4/p53 also activated transcription from collTRECAT although Gal4/p53 was much less efficient. Collectively, our data support a model where AP1 DNA binding sites recruit Gal4 fusion proteins to the promoter. Although the Gal4 moiety seemed to be sufficient for the tethering to AP1 DNA binding sites (see below), the presence of an activating domain linked to Gal4 was required for transcriptional activation. We have no explanation for the observed difference in relative activation between Gal4/CR1, Gal4/VP16 and Gal4/p53 on the two reporters.

Transactivation by the Gal4/CR1 fusion protein requires an AP1 factor containing c-Jun

Whereas Gal4/CR1 efficiently activated transcription from collTRECAT, Gal4(1–147), consistently repressed basal transcription from this reporter (Fig. 3A). Transcription from collTRECAT can be induced, either by cotransfection of CMVc-jun or by treating cells with the phorbol ester TPA (12-0-tetradecanoyl phorbol 13-acetate) (34). Significantly, Gal4(1–147) was also able to inhibit transcriptional activation by either c-Jun or TPA (Fig. 3A). Together, these data suggested that the Gal4 DNA binding domain could either recognize the AP1 DNA binding site directly, or interact with an AP1 transcription factor.

As shown in Figure 3A, TPA treatment induced transcription from collTRECAT ~10-fold, whereas Gal4/CR1 transfection resulted in an ~60-fold activation. If Gal4/CR1 would bind directly to the AP1 DNA binding site an antagonistic effect of TPA and Gal4/CR1 would most likely have been observed. However, TPA treatment of cells transfected with Gal4/CR1 induced CAT activity more than 1000-fold (Fig. 3A). This suggested that the Gal4/CR1 fusion protein recognized a DNA bound AP1 factor rather that the AP1 DNA binding site directly.

Jun is a common member of the AP1 transcription factor. Therefore, the direct involvement of c-Jun in the Gal4/CR1 transactivation of AP1 DNA binding sites was investigated in a cell line lacking c-Jun expression (A14 cells) (35). As can be seen

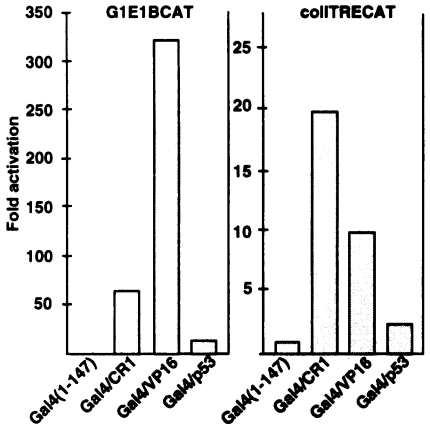

Figure 2. Different transactivation domains transactivate AP1-driven transcription when fused to Gal4(1–147). G1E1BCAT or collTRECAT were cotransfected into Hela cells and compared for their ability to be activated by the Gal4 fusion proteins Gal4/CR1, Gal4/VP16 and Gal4/p53. The graph shows mean induction of CAT activity from at least three independent experiments.

in Figure 3B, Gal4/CR1 transactivation of collTRECAT in A14 cells was severely reduced compared to transactivation in HeLa cells (Figs 1A and 3A). Transfection of CMVc-jun did not increase CAT activity from collTRECAT more than 2-fold. However, cotransfection of Gal4/CR1 and CMVc-jun gave an ~3-fold higher activity than with Gal4/CR1 alone. Although the cotransfected c-Jun was very poor, concerning transactivation, it most likely was able to bind to the AP1 DNA binding site and thereby serve as a docking factor for Gal4/CR1.

A direct protein/protein interaction between c-Jun and Gal4 through their DNA binding motifs

To directly test the possibility that Gal4 and a component of AP1 made physical contact, the Gal4(1–147) and Gal4/CR1 were expressed as GST fusion proteins in bacteria and thereafter challenged with *in vitro* translated c-Jun or Fos proteins. Ten per cent of the input of *in vitro* translated c-Jun was recovered from both the GSTGal4(1–147) and GSTGal4/CR1 agarose beads (Fig. 4A). The efficiency of c-Jun binding was independent of factors present in whole cell extracts (data not shown). In contrast, a weak binding of *in vitro* translated Fos to GSTGal4(1–147) was observed in whole cell extracts. This binding was almost completely lost if the HeLa cell whole cell extracts were omitted (data not shown). No binding to GST alone was detected (Fig. 4A).

To determine which part of c-Jun was required for the interaction with Gal4(1–147), two truncated forms of c-Jun were translated *in vitro*. The amino-terminal portion of c-Jun (Jun-TAD), expressing the activating domain, was not able to bind to GSTGal4(1–147) or GSTGal4/CR1 (Fig. 4A). In contrast, the carboxy-terminal portion of c-Jun (Jun-bZIP), containing the basic region and the leucine zipper, bound to GSTGal4(1–147) as efficient as full-length c-Jun (Fig. 4B).

To investigate which part of the Gal4 moiety was required for binding to the bZIP domain, two variants of GSTGal4(1–147), carrying deletions in the Gal4 region, were constructed. GSTGal4(1–74) retains the zinc-finger containing DNA binding domain and a weak dimerization element (36). GSTGal4 (74–147) lacks the DNA binding domain and has a truncated dimerization region (36). As shown in Figure 4B, both

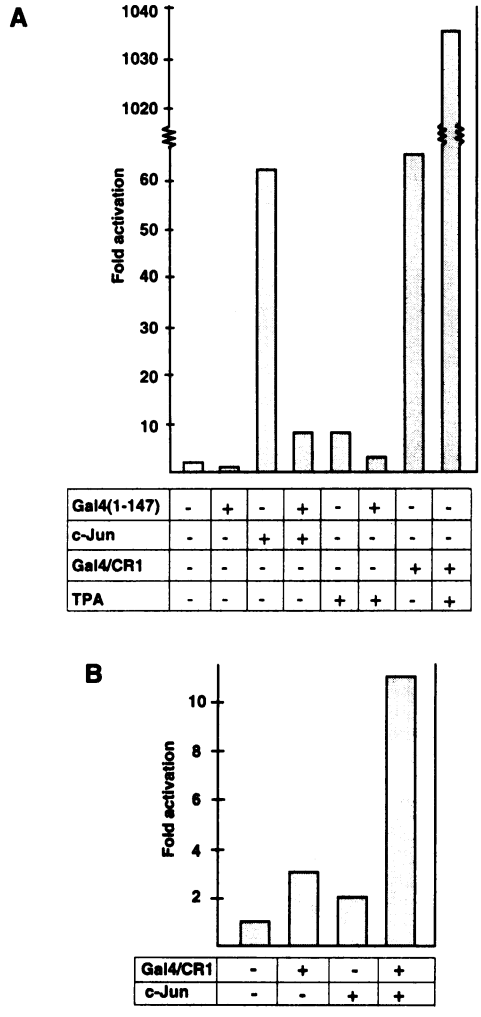

Figure 3. Transactivation by Gal4 fusion proteins require c-Jun. (**A**) Hela cells were cotransfected with the reporter plasmid collTRECAT and the indicated expression plasmids. Where indicated, 50 ng/ml TPA was added ~15 h before harvest. The panel shows the mean value of induction of CAT activity from at least three independent experiments. The left-most bar represents the expression from the reporter construct in the absence of any effector constructs, and has been given a relative expression value of 1. (**B**) Gal4/CR1 transactivation is impaired in A14 cells, a cell-line lacking endogenous c-Jun. Cotransfection and CAT analysis were as described in Figure 3A.

GSTGal4(1–74) and GSTGal4(1–94), but not GSTGal(74–147), could support the interaction with *in vitro* translated c-Jun. In addition, the bZIP domain of c-Jun was sufficient to bind to GSTGal4(1–74) and GSTGal4(1–94) (Fig. 4B).

To demonstrate that this region of Gal4 was also of importance for the observed ability of Gal4 fusion proteins to activate AP1 dependent transcription *in vivo*, the Gal4 deletions were tested in a Gal4/CR1 background. As can be seen in Figure 4C, Gal4(79–147)/CR1 was completely deficient for activation through AP1. On the other hand, Gal4(1–74)/CR1 retained ~10% of the activity of the parental Gal4/CR1. Approximately the same amount of proteins were expressed from all Gal4 deletion mutants (data not shown). From these results we conclude that the DNA binding domains of Gal4 and c-Jun can interact both *in vivo* and *in vitro* and furthermore, that this interaction may explain the ability of Gal4 fusion proteins to activate AP1 dependent transcription.

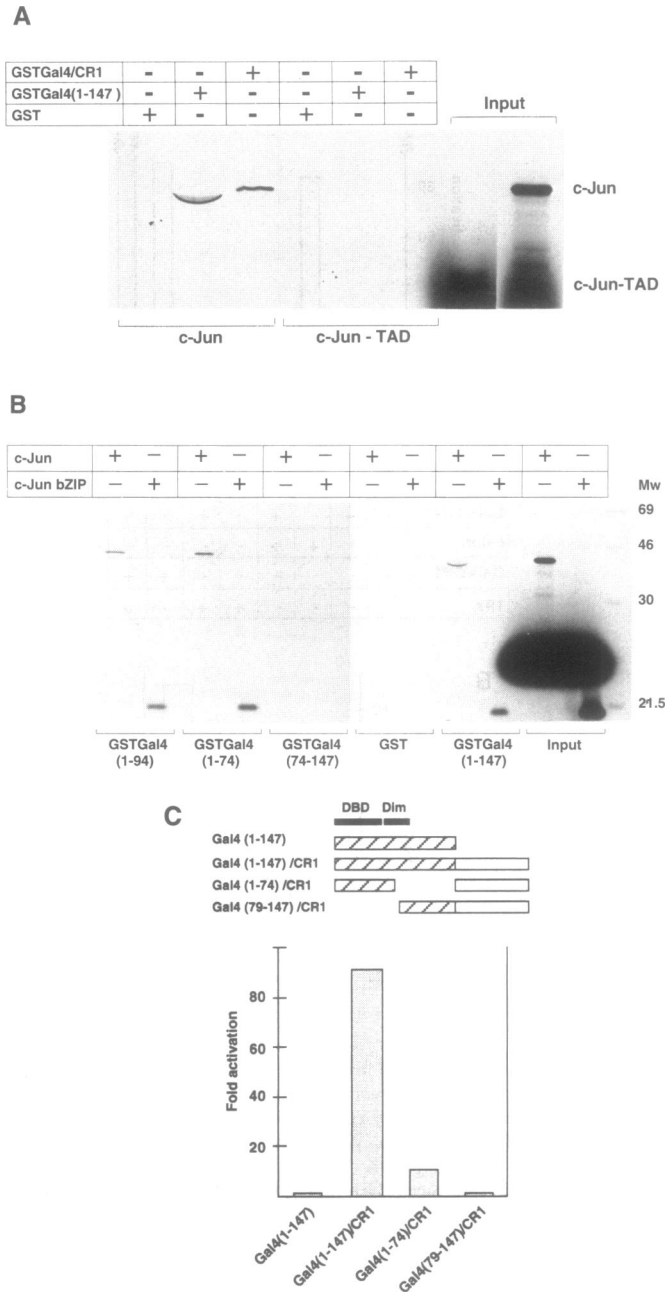

Figure 4. The Gal4 and c-Jun proteins can physically interact. (**A**) Full-length c-Jun, but not the transactivation domain alone, can bind to Gal4(1–147) *in vitro*. c-Jun or a truncated protein containing the first 244 amino acids of c-Jun (Jun-TAD) was produced by *in vitro* translation in the presence of ^{35}S methionine and bound to bacterially produced GSTGal4(1–147) or GSTGal4/CR1. Input represents equal amounts of unbound, *in vitro* translated c-Jun proteins. The dark spot most likely represents ^{35}S methionine charged tRNA. Following electrophoretic separation bound proteins were visualized by autoradiography. (**B**) Interaction between the DNA binding domains of c-Jun and Gal4. The full length c-Jun or the C-terminal part (starting at amino acid 247) of c-Jun (Jun-bZIP) were expressed *in vitro* and bound to the indicated GSTGal deletion mutants as described in A. (**C**) The DNA binding domain of Gal4 is required for transactivation by Gal4/CR1. Hela cells were cotransfected with collTRECAT and indicated Gal4 deletion mutants. A schematic presentation of the different Gal4 constructs is shown at the top. The DNA binding (amino acids 1–65) and dimerization (amino acids 65–94) domains of Gal4 are indicated. Gal4(1–147)/CR1 is identical to Gal4/CR1. The graph shows the mean value of induction of CAT activity from at least three independent experiments.

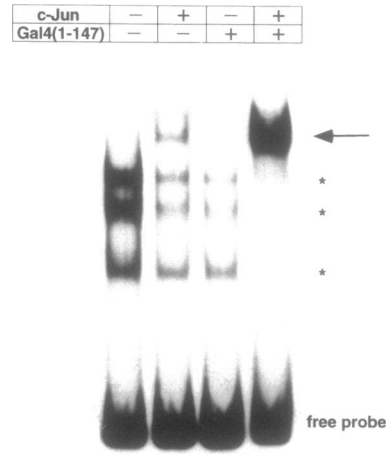

Figure 5. Gal4(1–147) increases binding of c-Jun to a consensus AP1 DNA binding site. c-Jun and Gal4(1–147) were produced by *in vitro* translation either separately or simultaneously as indicated in the panel. Following binding to the ^{32}P-labelled DNA fragment containing a dimerized AP1 DNA binding site, the complexes were separated on a native polyacrylamide gel. Protein–DNA complexes were visualized by autoradiography. Unspecific band-shifting activities resulting from the *in vitro* translation extracts are marked by asterisks. The specific c-Jun induced shift is marked by an arrow.

Increased binding of c-Jun to AP1 binding sites in the presence of Gal4

Our data are consistent with a model where Gal4 fusion proteins are recruited to a promoter through a DNA bound AP1 factor. However, we have not been able to isolate an *in vivo* Gal4–c-Jun–DNA complex. When *in vitro* translated c-Jun and Gal4(1–147) were mixed with a ^{32}P-radiolabelled DNA fragment containing AP1 DNA binding sites, Gal4(1–147) stimulated Jun–DNA complex formation ~10-fold (Fig. 5). However, no change in electrophoretic mobility, indicative of the presence of Gal4(1–147) in the complex was observed (Fig. 5). It is possible that Gal4(1–147) stimulates Jun–DNA complex formation, but is then dissociated during the electrophoretic separation.

Putative AP1 DNA binding sites in the plasmid G1E1BCAT vector sequence serve as targets for activation by a Gal4 fusion protein

The G1E1BCAT reporter, constructed by Lillie and Green (1988), harbours a pUC18 derived sequence in an otherwise pSP72 background. In contrast, the E1BTATACAT reporter, constructed by the same group, has no pUC18 derived sequences. The DNA sequence of the pUC derived fragment contains several putative AP1 DNA binding sites. One of these sites has indeed been shown to confer transcriptional activation by Jun or Jun/Fos (37). Reciprocal exchanges of vector sequences between G1E1BCAT and E1BTATACAT, creating G1E1BCATΔ (lacking pUC sequences) and pUC-E1BCAT (supplemented with the pUC derived fragment), revealed that the pUC derived fragment conferred both c-Jun and Gal4/CR1 inducibility (Fig. 6). Furthermore, Gal4/CR1 activation of a reporter containing one Gal4 binding site was several fold higher in the presence (G1E1BCAT), than in the absence (G1E1BCATΔ), of the pUC

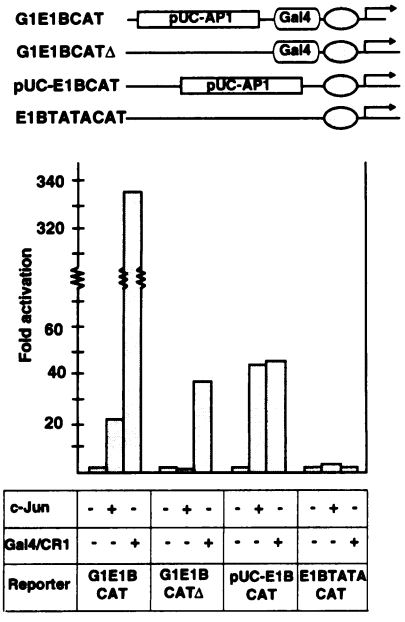

Figure 6. Vector-derived AP1 DNA binding sites in G1E1BCAT mediate transactivation by Gal4/CR1 and c-Jun. Hela cells were cotransfected with the reporter constructs depicted at the top, and CMVc-jun or Gal4/CR1. Hatched circles represent the adenovirus E1B TATA box. pUC-AP1 represent a pUC vector sequence derived from the backbone of G1E1BCAT. The graph shows the mean value of induction of CAT activity.

derived fragment (Fig. 6). On the basis of these observations, we suggest that it is unsuitable to use G1E1BCAT as a single binding site reporter since it functionally may recruit (at least) two Gal4 fusions protein dimers.

DISCUSSION

In this paper we show that the yeast Gal4 transcription factor can functionally interact with the mammalian AP1 transcription factor. *In vitro*, the interaction was dependent on the zinc finger region of Gal4 and the bZIP domain of c-Jun. The bZIP domain of c-Jun has been shown to interact with the zinc finger containing DNA binding motif of the glucocorticoid receptor (GR) (11–13,15). The zinc fingers of Gal4 and GR belong to different classes of zinc finger proteins, but share similarities concerning their co-ordinate binding of two zinc ions exclusively through the usage of conserved cysteines (14,20 and references therein). The interaction between Jun and GR has been shown to exclude GR binding to its DNA binding element and to reduce AP1 binding to AP1 DNA binding sites (11,13,15). Cotransfected CMVc-jun did not cause any significant reduction in the level of Gal4/CR1 transactivation from Gal4 binding sites (data not shown). Therefore, we find it unlikely that the Gal4–c-Jun interaction prevents binding to Gal4 binding sites. Neither do we believe that Gal4 can prevent AP1 binding to AP1 binding sites based on the following observations; whereas Gal4(1–147) alone efficiently repressed c-Jun or TPA induced AP1 dependent gene expression *in vivo*, a Gal4 fusion protein encoding an activating domain induced AP1 dependent transcription (Figs 1 and 2). The absolute requirement for an activator linked to Gal4 when scoring for transactivation through AP1 factors are difficult to reconcile with any other model than a direct recruitment of the Gal4 fusion protein to a DNA bound AP1. Furthermore, *in vitro*, Gal4(1–147) increased binding of c-Jun to the AP1 DNA binding site (Fig. 5). Importantly, association between Gal4 and c-Jun *in vitro* was completely unaffected by the presence or absence of the activating domain (Fig. 4A). In summary, we propose the following scenario for the effect of Gal4 on AP1 dependent transcription. The endogenous AP1 activity in HeLa cells mainly consists of Jun/Jun homodimers with a relatively poor ability to induce TRE driven transcription. The DNA binding domain of Gal4 can bind Jun and thereby increase the affinity of Jun/Jun homodimers to TRE. At the same time, this interaction masks the transactivation domain of Jun, explaining the observed Gal4 repression of c-Jun induced transcription. A Gal4 fusion protein, carrying a transactivation domain, may on the other hand, substitute for the non-functional activating domain of Jun, and thereby cause the observed transactivation.

We have not been able to demonstrate an *in vivo* complex containing Gal4 and c-Jun. This may not be surprising, since several other groups have presented difficulties in isolating complexes between Jun and members of the steroid hormone receptor family *in vivo*, unless UV or chemical cross-linking were performed (9,13,16)

Using an electrophoretic mobility shift assay, the addition of Gal4(1–147) resulted in a 10-fold increase in the amount of Jun–DNA complex (Fig. 5). However, no change in mobility was observed that could indicate the presence of the Gal4(1–147) factor in the complex. The HTLV-I Tax protein can stimulate the binding of bZIP proteins to DNA by increasing formation of homodimers (38,39). Similar to our own results (Fig. 5), the presence of Tax induced increased binding of bZIP proteins to a DNA probe in mobility shift assays, but did not alter the electrophoretic mobility, probably due to instability of the complex [(39) and references therein].

In vitro translated Fos could also bind to GSTGal(1–147), although less strong, and primarily in the presence of HeLa whole cell extracts. It has been reported that GR can interact both with Jun and Fos, although the interaction with Fos was much weaker (12). Since Fos is unable to homodimerize this may indicate that Fos monomers are unable to bind to Gal4, but that heterodimers between Jun and Fos will. We are currently investigating the ability of Gal4 fusions to activate AP1 dependent transcription in cells expressing both Jun and Fos.

We describe here the ability of a yeast transcription factor to physically interact with the mammalian AP1 transcription factor. Although there is no known mammalian homologue to Gal4, c-Jun shows homology with the yeast factor GCN4 (40). It is therefore possible that a cross-talk between Gal4 and GCN4 exists in yeast, and if so, the bZIP–zinc finger interaction would be a more general phenomenon.

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